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Granzyme B is expressed in urothelial carcinoma and promotes cancer cell invasion

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Granzyme B (GrB) is a serine proteinase known to be expressed by cytotoxic lymphocytes and to induce, in presence of perforin (Pf), apoptosis in target cells. Recently, GrB expression has been shown (often in absence of Pf) in nonlymphoid cells, but its function is not defined. In our study, we investigated GrB and Pf expression in bladder cancer cell lines and in urothelial carcinoma (UC) tissues by reverse transcription-polymerase chain reaction (RT-PCR), Western blot, ELISA, immunofluorescence and immunohistochemistry. We also assessed the function of GrB in UC cells; the *in vitro* function of GrB was examined by loss-of-function experiments. Our results revealed that GrB is expressed, in absence of Pf, in UC cells. Significant differences were found between GrB expression and both increasing pathological tumor spreading and high-grade *vs.* low-grade pTa tumors. Notably, GrB in UC tissues was concentrated at the cancer invasion front and was expressed in neoplastic cells undergoing epithelial-mesenchymal transition, a key event in carcinoma invasion. Indeed, GrB-positive cells also expressed Snail, N-cadherin or were negative for E-cadherin. GrB expressed in tumor cell lines was enzymatically active and capable of vitronectin cleavage, implying extracellular matrix (ECM) remodeling by GrB. Inhibition of GrB activity or Stealth RNA interference-mediated GrB gene silencing markedly suppressed bladder cancer cell invasion through matrigel. This data provides the first evidence for a role of GrB in promoting cancer cell invasion. Taken together, our findings suggest that GrB, *via* ECM degradation, contributes to the establishment of the UC invasive phenotype.

Urinary bladder cancers are the 4th most common cancer and the 5th leading cause of cancer death among males in Western countries. The majority (>90%) of bladder cancers are urothelial carcinomas (UCs).[1] More than 70% of the incidence is due to papillary noninvasive UCs, that recur in >50% of patients but progress to invasive disease only infrequently.[1] Most of the mortality, on the other hand, occurs in the other 20–30% of patients who present with invasive UCs, characterized by a high metastatic rate.[1] A more extensive understanding of the molecular mechanisms underlying UC invasion, the first event in metastasis, is needed to find molecular markers predicting disease progression and to develop improved therapeutic targets for treatment of invasive UCs.[2–4]

Granzyme (Gr) B is a serine proteinase contained, together with other Grs and perforin (Pf), in cytoplasmic granules of activated cytotoxic T lymphocytes (CTLs) and natural killer (NK) cells.[5,6] On effector-target cell interaction, GrB is exocytosed and delivered by means of Pf inside the cytosol of target cells, where it induces apoptosis *via* proteolysis of intracellular substrates.[7–9]

Although data in the literature should be carefully interpreted because of GrB interspecies functional diversity,[8–13] accumulating evidence suggests that human GrB possesses, besides the apoptotic intracellular role, extracellular function.[13–17] Recently, extracellular matrix (ECM) remodeling *via* cleavage of vitronectin, fibronectin and laminin has been reported by human GrB.[15] Different consequences derived from this activity have been assumed, including the possible contribution of GrB to cellular tissue infiltration, but this idea has never been tested and the biological relevance of ECM proteolysis by GrB has not yet been elucidated.[13,16,17]

The expression of Grs, initially thought to be restricted to lymphoid cells,[5,6,18] is known today to be more extensive.[7,8]

Key words: granzyme B, urothelial carcinoma, invasion, extracellular matrix, epithelial-mesenchymal transition

Abbreviations: CTL: cytotoxic T lymphocytes; ECM: extracellular matrix; EMT: epithelial-mesenchymal transition; GrB: granzyme B; NK: natural killer; Pf: perforin; RNAi: RNA intereference; UC: urothelial carcinoma

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